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TITLE: Developing Novel PepT1-Targeted Modulators for  
Inflammatory Bowel Disease (IBD) Therapy

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| 14. ABSTRACT<br>The overarching research goal of this project is to contribute to the understanding of inflammatory bowel disease (IBD) mechanisms by providing an integrated experimental and computational description of substrate specificity in the human Proton-coupled oligopeptide transporter, PepT1 (SLC15A1). The methodologies include structure-based drug discovery computations, combined with cell-based and in vivo models of IBD. During the past year, we have made significant progress toward completing our Project Goals. We have continued to characterize structure-function relationships in PepT1. IN particular, we (i) developed structural models for PepT1 in different conformations, (ii) predicted small molecule ligands using virtual screening, and (iii) tested the model and ligands experimentally using the yeast <i>P. pastori</i> , to refine the initial models and optimize ligands. These results confirm the relevance of the PepT1 model for drug discovery as well as reveal previously unknown chemical scaffolds that interact with this transporter. Furthermore, our results provide a framework for developing more potent ligands in various cellular and animal IBD models, directly addressing Aims 2 and 3 of our Project Goals. |              |                          |                            |   |   |
| 15. SUBJECT TERMS<br>Homology modeling, Virtual screening, Membrane protein, Proton-coupled oligopeptide transporter (POT), SLC15, PepT1, Docking, Transgenic mouse, IBD   |              |                          |                            |   |   |
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## 1. INTRODUCTION

The two major forms of inflammatory bowel disease (IBD), Crohn's disease and ulcerative colitis, are chronic disorders characterized by nonspecific inflammation and intestinal tissue damage. IBD incidence and prevalence have been increasing with time and in different regions around the world, indicating its emergence as a global disease, particularly of the Western Society and Northern America. Current IBD treatments involve anti-inflammatory drugs, immunosuppressants, biologic agents and antibiotics, as well as drugs for symptomatic relief. PepT1 is a protein that is primarily found in the small intestine where it absorbs dietary degradation products and therapeutic agents such as antibiotics and antiviral drugs. Recent studies suggest that PepT1 is also present in the colon of IBD patients and plays a key role in inflammation by transporting bacterial-derived products into the intestinal cells. PepT1 is, therefore, an important and novel IBD drug target, in which PepT1 inhibitors can be used to block the uptake of harmful peptides of bacterial origin, or PepT1 substrates to efficiently deliver anti-inflammatory agents to problematic intestinal cells. The goal of this project is to discover new chemical tools to modulate and explore PepT1 function, using a structure-based discovery approach and transgenic mice models, with a long-term objective of developing innovative medicines for IBD.

## 2. KEYWORDS

Homology modeling, Structure-based drug discovery, Virtual screening, SLC, Membrane protein, Proton-coupled oligopeptide transporter (POT), SLC15, PepT1, Docking, Transgenic mouse.

## 3. ACCOMPLISHMENTS

### ➤ What were the major goals of the project?

The overarching research goal of this project is to contribute to the understanding of inflammatory bowel disease (IBD) mechanisms by providing an integrated experimental and computational description of substrate specificity in the human Proton-coupled oligopeptide transporter, PepT1 (SLC15A1). The methodologies include structure-based drug discovery computations, combined with cell-based and *in vivo* models of IBD. Our Specific Aims are:

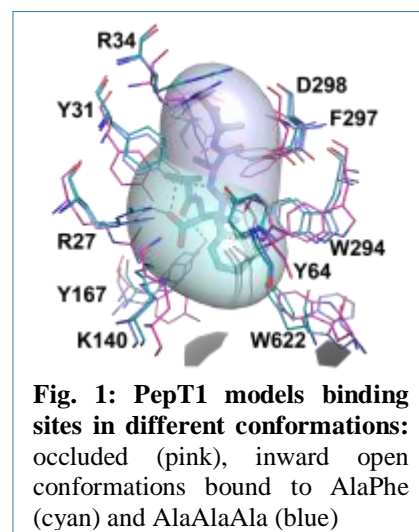
1. Develop structural models for PepT1 in different conformational states, using homology modeling and Molecular Dynamics simulations.
2. Discover small molecule ligands for PepT1, using virtual screening followed by experimental testing.
3. Rationally refine PepT1 transport modulators and establish the importance of PepT1 and its ligands in IBD.

### ➤ What was accomplished under these goals?

During the past year, we have made significant progress toward completing our projects' goals. We have continued to characterize structure-function relationships in PepT1. Several new structures of homologs of this protein have been recently determined, providing new templates for our modeling of the human PepT1. In fact, our labs have been invited to write a commentary in *Cell Chemical Biology* regarding these new advancements in the field (Colas *et al* 2016). The major accomplishments for this project include:

### ✓ Develop structural models for PepT1 in different conformations.

We have developed homology models for PepT1 in four different conformations relying on structures of various prokaryotic homologs as modeling templates (Fig. 1). Two of the models are based on the occluded structure of PepT<sub>So</sub> (PDB identifier 2XUT) and inward-open conformation of PepT<sub>Gk</sub> (ligand bound structure; PDB identifier 4IKZ). These models propose key binding and transport specificity determinants. For example, the inward-open PepT1 model bound to the substrate Alafosfalin, reveals important residues for ligand coordination (e.g. Y31 and R27). We also modeled PepT1 based on inward-open conformations of PepT<sub>Si</sub> bound to a di- and tripeptides (PDB identifiers 4D2C and 4D2D, respectively). These models show distinct mode of interactions for di- and



**Fig. 1: PepT1 models binding sites in different conformations:** occluded (pink), inward open conformations bound to AlaPhe (cyan) and AlaAlaAla (blue)

tripeptides as well as unique conformations of PepT1 binding site when bound to each ligand type. Notably, these models provide a framework to discover potent inhibitors for this protein in Aim 2 of our project.

✓ *Predict small molecule ligands using virtual screening.*

We performed multiple virtual screenings against the PepT1 models, using the lead-like and fragment libraries of the ZINC15 database of small molecules. We took a variety of considerations to prioritize molecules for experimental testing, including the docking score and chemical novelty of the predicted ligands. Furthermore, because erroneous docking may occur in structure-based virtual screenings, we carefully inspected the docking pose of these top-ranking compounds, to remove molecules with a questionable pose or strained conformation. Finally, we focused on molecules that form hydrogen bonds with the conserved polar residues of the ligand binding site (i.e., Y31), as well as van der Waals interactions with residues that form a hydrophobic subpocket in the binding site (e.g., with W622) (Fig. 2).

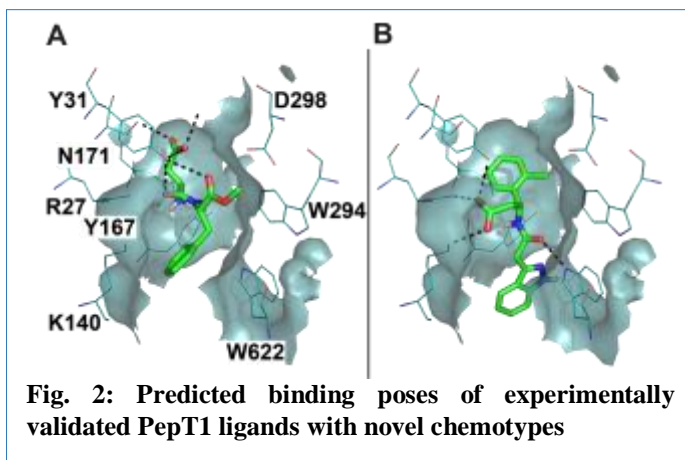


Fig. 2: Predicted binding poses of experimentally validated PepT1 ligands with novel chemotypes

✓ *Test the model and predicted ligands experimentally using the yeast *P. pastoris*; Refine the initial models and optimize ligands.*

We established the yeast *Pichia pastoris* recombinant expression host for the human PepT1. We then performed *cis*-inhibition assays against cefadroxil to determine IC<sub>50</sub> values. Of the 22 tested compounds five were confirmed as PepT1 ligands. As new ligands were discovered by us and others, we iteratively refine our structural models, to improve its predictive power. These results confirm the relevance of the PepT1 model for drug discovery as well as reveal previously unknown chemical scaffolds that interact with this transporter.

➤ **What opportunities for training and professional development has the project provided?**

One postdoc from the Schlessinger lab, Dr. Claire Colas, has been trained in this project. In brief, Dr. Colas has an excellent background in computational chemistry. In the past year, Dr. Colas has honed her skills in state-of-the-art homology modeling and virtual screening methods, and understanding membrane transport. Claire and Dr. Schlessinger have developed a plan for her to improve her ability to perform the various activities needed from an independent researcher. Particularly, we have focused on working on her technical writing and presentation skills. For example, Claire has helped writing the commentary paper published earlier this year in *Cell Chemical Biology*, which is directly relevant to this project. Furthermore, Claire gave oral presentations in two important symposia during this reporting period: An international symposium about membrane transporters at the New York Academy of Science and the ACS North East Regional Meeting in 2016. She also presented posters in other symposia.

In addition, the Office of Postdoctoral Affairs in Mount Sinai provides many resources including a career counselor who can assist postdocs in developing Individual Development Plans (IDPs). The PI and postdoctoral fellow fill together an annual mandatory Postdoctoral Evaluation Form. This form includes questions about progress in professional development, as well as strengths and weaknesses of the postdoc.

➤ **How were the results disseminated to communities of interest?**

The results have been disseminated through invited presentations and published papers (see below).

➤ **What do you plan to do during the next reporting period to accomplish the goals?**

In the next reporting period (six month) we will focus on the following three research directions: First, we will complete writing our manuscript describing the PepT1 models and mode of interactions with the newly discovered ligands. Second, we will continue characterizing new modes of interactions between PepT1 and small molecules, and refine our initial hits to develop structure-activity relationship (SAR) models. These

optimized compounds will be tested in various cellular and animal IBD models. These studies directly address Aims 2 and 3 of our Project Goals.

#### 4. IMPACT

➤ **What was the impact on the development of the principal discipline(s) of the project?**

Our integrated approach enabled the visualization of the human PepT1 binding site in different conformations for the first time, contributing to the understanding of PepT1 function. For example, we propose that specific interactions between PepT1 and small molecules determine whether a molecule is likely to be an inhibitor that binds the transporter or a substrate that gets transported into the cell. In addition, we discovered chemically unique compounds that inhibit transport by PepT1 by interacting with previously unknown subpockets on the protein surface. This result provides a starting point for the development of lead molecules as putative IBD therapeutics.

➤ **What was the impact on other disciplines?**

While the goal of this project was to identify novel PepT1 modulators as potential IBD drugs, PepT1 also has key pharmacological and physiological roles. PepT1 is found in the small intestine where it absorbs dietary degradation products and therapeutic agents such as antibiotics and antiviral drugs and it is also a putative drug target for other diseases (e.g., pancreatic cancer). Therefore, the new tool compounds we identified can be used to design compounds with optimal intestinal absorption *via* PepT1 or target other diseases such as cancer, similarly to targeting this protein for treating IBD.

In addition, we established a pipeline that combines computational structural biology and cell-based assays to identify useful chemical probes modulating the human PepT1 at a high hit-rate. This integrated pipeline is generally applicable to the majority of the human SLC transporters (about 350 proteins) including drug targets and transporters that regulate drug absorption, distribution, metabolism, and excretion. Finally, our studies address a basic question in biophysics and cell biology: how are oligopeptides recognized by membrane transporters and transported into the cell?

➤ **What was the impact on technology transfer?**

Nothing to Report

➤ **What was the impact on society beyond science and technology?**

Nothing to Report

#### 5. CHANGES/PROBLEMS

Our structural models of PepT1 were sufficiently accurate for structure-based virtual screening at a high hit-rate. Thus, despite the fact that in our original proposal we proposed to confirm the models using site directed mutagenesis, we decided to invest our efforts in characterizing the newly discovered ligands (e.g., by electrophysiology) instead of performing mutagenesis. We expect this minor change to expedite our progress toward animal model testing. Finally, no changes occurred or will occur in the use and care of vertebrate animals, biohazards and/or select agents.

#### 6. PRODUCTS

##### **Publications**

- Colas C, Smith DE, Schlessinger A (2016) Computing Substrate Selectivity in a Peptide Transporter *Cell Chem Biol* 23 (2):211-3
- Colas C, Ung PMU, Schlessinger A (2016) SLC Transporters: Structure, Function and Drug Discovery *Med Chem Comm* 7:1069-81

##### **Presentations**

###### Avner Schlessinger,

- NCCR-Transcure, Bern, Switzerland. Sep 14, 2016
- 252th ACS National Meeting, Philadelphia, PA. Aug 21, 2016
- Special Symposium on Solute Carriers in New York Academy of Science, New York, NY. Apr 26, 2016
- Showcase Award, ASPET Annual Meeting at Experimental Biology 2016, San Diego, CA. Apr 4, 2016

- Laufer Center for Physical and Quantitative Biology at Stony Brook University, NY. Mar 25, 2016
- 251th ACS National Meeting, San Diego, CA. Mar 15, 2016
- Dept. of Chemistry and Biochemistry, Worcester Polytechnic Institute, Worcester, MA, Sep 16, 2015
- Dept. of Bioinformatics, Technical University of Munich, Munich, Germany, Sep 10, 2015
- Transmembrane Transporters in Health and Disease, 8th SFB35, Vienna, Austria, Sep 8, 2015

#### Claire Colas

- ACS North East Regional Meeting, Binghamton, NY. Oct 6, 2016
- Special Symposium on Solute Carriers in New York Academy of Science, New York, NY. Apr 26, 2016

### **7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

Avner Schlessinger, PhD (Initiating PI) – No change

David Smith, PhD (Partnering PI)- No change

Claire Colas, PhD – No change

### **8. SPECIAL REPORTING REQUIREMENTS**

Both Initiating and Partnering PI uploaded the same report.

### **9. APPENDICES**

None.